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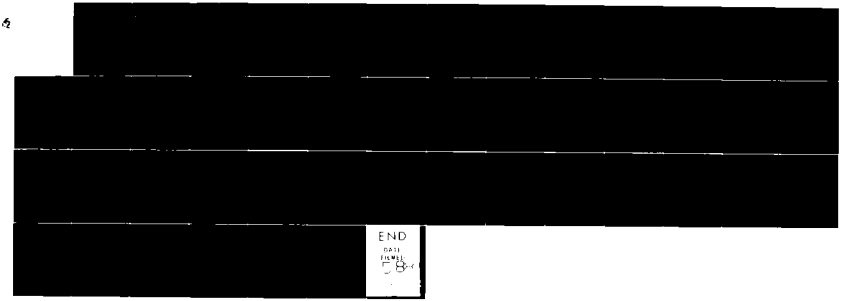

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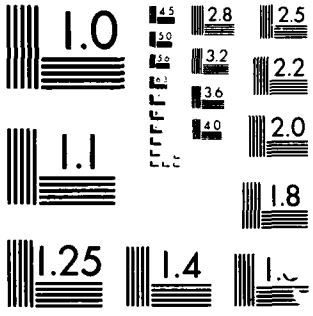

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SYNTHESIS OF IMPROVED ANTILEISHMANIAL AND ANTITRYPANOSOMAL DRUGS

ANNUAL AND FINAL REPORT

By

Anica Markovac
Maurice P. LaMontagne
Dinesh J. Dagli
Geng-Shuen Wu
M.S. Khan
Arthur B. Ash
Calvin L. Stevens

August 1984

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ASH STEVENS INC.
Detroit Research Park
5861 John C. Lodge Freeway
Detroit, Michigan 48202

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Antileishmanial drugs	Bis(amidoximes)	Synthesis
Antitrypanosomal drugs	Bis(amidines)	HOE 668
8-Aminoquinolines	Styrylbenzimidazoles	Pentamidine
2,6-Bis(phenyl)pyridines	Styrylpyridines	Stilbamidine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The program was directed at the preparation of improved antileishmanial and antitrypanosomal drugs. Over the six and one-half year duration of the contract, 108 compounds - 56 target candidate drugs and 52 intermediates - were submitted for biological testing. The program was divided into five phases of work (number of compounds in paren.): analogs of WR 6026, 8-[(6-diethylhexyl)amino]-6-methoxy-4-methylquinoline (15), 7-aminoquinolines (3), 3-aminoquinolines (2) and 4-amino-2,6-substituted-pyridines (1), aryl/heterocyclic bis(amidoximes)

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20. ABSTRACT (Continued)

and bis(amidines) (20), bis(amidoximes) of four clinical bis(amidines) (8) and HOE 668 and structural modifications (7). Biological data available to date indicate that promising new leads have been developed against L. donovani and T. rhodesiense.

FOREWORD

The work described herein was performed under Contract No. DAMD17-78-C-8001 for the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Walter Reed Army Medical Center. This report covers the period of 1 October, 1977 through 31 March 1984.

ACKNOWLEDGEMENT

The work was performed under the general direction of C.L. Stevens, Principal Investigator. A. Markovac served as Associate Investigator, succeeding M.P. LaMontagne who served through June, 1981. D.J. Dagli, Geng-Shuen Wu and M.S. Khan served as Senior Research Chemists and A.B. Ash as Program Manager. The timely advice, encouragement and assistance of H.A. Musallam and E.A. Steck is gratefully acknowledged. H.A. Musallam succeeded E.A. Steck who served as COTR through January 15, 1981.

TABLE OF CONTENTS

SYNTHESIS OF IMPROVED ANTILEISHMANIAL AND ANTITRYPANOSOMAL DRUGS

1. INTRODUCTION AND BACKGROUND

The present contract by Ash Stevens Inc. was intended to provide synthesis support for the research effort against leishmaniasis and trypanosomiasis by the U.S. Army Medical Research and Devlopment Command. This program which was gauged to a one-man level of effort plus supervision and technician assistance was designed to assess the potential of classes of compounds which have displayed antiparasitic activity. The objective was to acquire superior drugs relative to current antileishmanial and antitrypanosomal experimental and clinical drugs. The importance of the program to the Army is illustrated by the fact that 50% of Israeli forces operating in the Jordan Valley during the "Six Day War" were infected with leishmaniasis (Kinnamon, et al., ref. 1); additionally leishmaniasis affects well over 10 million people world wide (Berman, 1981, ref. 2).

The recognized group of four diseases caused by protozoal parasites (haemoflagellates) belong to the family Trypanosomatidae and the genus Leishmania: Leishmania donovani, L. brasiliensis, L. tropica and L. mexicana. The army is extensively involved in both the biological and therapeutic aspects of the diseases generated by the first three of these parasites (Davidson, ref. 3). The most lethal form of the disease is visceral leishmaniasis (kala-azar) which is generated by L. donovani and is fatal in 98% of untreated cases (ref. 1). To evaluate new candidate drugs, the Army provides a test screen developed by W.L. Hanson (ref. 4) based on L. donovani in the golden hamster in which the drug is administered IM or SC and, if warranted, PO. The standard for comparison is the clinically effective drug, glucantime^{R}, an antimonial. Thus, the glucantime^{R} index (G) is the ratio of SD_{90} for glucantime/SD_{90} for the new drug where SD_{90} = 90% suppression of parasites (dosage). The antimonial drugs are more toxic than desired. Certain bis(amidines) such as pentamidine and hydroxystilbamidine have served as clinical antileishmanial drugs even though the therapeutic indices and effectivity are lower than desired.

The second genus of the family Trypanosomatidae is the genus Trypanosoma. The Army provides a test system for T. rhodesiense (both SC and PO) in the mouse (Rane/Ager) and the more refractory T. cruzi (Chagas' disease) in the mouse (Ager) and the feedback of results from the T. rhodesiense test is quite rapid. Thus the candidate drugs are screened against one or two forms of both leishmaniasis and trypanosomiasis. Selected compounds are evaluated in the Rane P. berghei mouse antimalarial screen at three dose levels as well as in the causal prophylactic P. berghei yoelii mouse screen. The various strains and chemotherapy of trypanosomiasis (sleeping sickness) are well reviewed in Burger's Medicinal Chemistry, 4th Ed., p. 440, 1979 (ref. 5).

Over the years, in the search for more effective antileishmanial drugs the U.S. Army Medical R & D Command had screened large numbers of their antimalarial research drugs in the L. donovani hamster screen. This indicated that many of the simple 6-methoxy-8-aminolepidines were active in this screen and that the activity was very sensitive to the structure of the 8-aminoalkylamino side chain. The most active of these was WR 6026, 8-diethylaminohexylamino-6-methoxylepidine, which has a glucantime index of 474 (IM) and 708 (PO, ref. 6).

In view of the success of WR 6026 and related structures, the first major phase of work under this contract involved the preparation of WR 6026 analogs in which the 8-diamine side chain was varied (12 compounds). Additional substituents were placed in three other structures bearing the optimum 8-diethylaminohexylamino side chain to bring the total to 15 compounds. A certain degree of success was achieved in that four compounds had G indices of 300 to 333 (all IM) vs 474 for WR 6026 (1980, ref. 7) and some further work is recommended.

In a more limited second phase effort, three 7-aminoquinolines (ref. 8), a 4-aminopyridine and three 3-aminoquinolines were prepared, all bearing the optimum 6-diethylaminohexylamino side chain, but none showed significant antileishmanial (nor antitrypanosomal) activity.

In the third phase of work, the effort has been directed to the synthesis of aryl/heterocyclic bis(amidines) and the corresponding bis(amidoximes). This was extended in a fourth phase of work to the synthesis of four bis(amidoxime) analogs of the clinical drugs pentamidine, berenil, stilbamidine and 2-hydroxystilbamidine. Finally, as a fifth phase of work, analogs of the highly active (but hepatotoxic) HOE 668 were prepared.

As both Peters (1975, ref. 9) and Steck (ref. 10) have noted, "certain tri- and pentavalent antimonials and diamidines have formed the backbone of the chemistry of leishmanials for many years". Peters commented later (1980, ref. 11) that

> "Very few leishmanicidal drugs are available for the therapy of visceral leishmaniasis in man or dogs. Two pentavalent antimonials provide the first-line treatment of choice, namely sodium stilbogluconate (Pentostam, 28-29.5% Sb^V), and meglumine antimoniate (Glucantime, 33% Sb^V). Pentamidine isethionate is sometimes used successfully when antimonials fail, but it is said to be of little value in East Africa."

The biological activity data acquired to date are presented in Section 2 following.

1.1 References Cited. Introduction.

1) "Leishmaniasis. Military Significance and New Hope for Treatment". K.E. Kinnamon, P.S. Louizeaux, V.B. Waits, E.A. Steck, L.D. Hendricks, W.L. Chapman and W.L. Hanson, Military Medicine, 144, 660 (1979).

2) J.E. Berman, Am. J. Trop. Med. Hyg., 30, 556 (1981).

3) D.E. Davidson, Jr., Personal Communication.

4) "Testing of Drugs for Antileishmanial Activity in Golden Hamsters infected with Leishmania donovani", W.L. Hanson, W.L. Chapman, Jr. and K.E. Kinnamon, International Journal for Parasitology, 7, 443 (1977).

5) "Burger's Medicinal Chemistry" Fourth Edition, Part II; M.E. Wolff, Ed., John Wiley and Sons, New York, 1979.

6) "The Antileishmanial Activity of Lepidines" K.E. Kinnamon, E.A. Steck, P.S. Louizeaux, W.L. Hanson, W.L. Chapman, Jr. and V.B. Waits, Am. J. Trop. Med. Hyg., 27, 751 (1978).

7) "Analogues of 8-[[6-(Diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline as Candidate Antileishmanial Agents", M.P. LaMontagne, D.J. Dagli, M.S. Khan and P. Blumbergs, J. Med. Chem. 23, 981 (1980).

8) "7-Aminoquinolines as Candidate Antiparasite Agents", A. Markovac, G.S. Wu, M.P. LaMontagne, P. Blumbergs and M.S. Ao, J. Heterocyclic Chem., 19, 829 (1982).

9) "The Experimental Chemotherapy of Leishmaniasis, II", N.W. Mattock and W. Peters, Annals of Tropical Medicine and Parasitology, 69, 359 (1975).

10) E.A. Steck, "The Chemotherapy of Protozoan Diseases", Volume II, Section 3, Division of Medicinal Chemistry, Walter Reed Army Institute of Research.

11) "The Experimental Chemotherapy of Leishmaniasis, V", W. Peters, E.R. Trotter and B.L. Robinson, ibid, 74, 289-298 (1980).

2. BIOLOGICAL RESULTS. ANTILEISHMANIAL AND ANTITRYPANOSOMAL ACTIVITY DATA.

The compound structures and antileishmanial data for all five phases of work will be presented first (section 2.1) followed by the antitrypanosomal data (section 2.2). Phase 1 and 2 compounds are useful only as leishmaniacides and their structure and biological data are combined in individual tables. On the other hand, the structures of Phase 3, 4 and 5 compounds are shown in three separate figures. The results are summarized in five tables for leishmanial data and three for trypanosomal data. The compounds are referenced by the ASI SNL number, and by the WR No. and/or the Bottle Number.

2.1 ANTILEISHMANIAL DATA (Phases 1,2,3,4 and 5)

Phase 1 Compounds. Analogs of WR 6026 (15). 8-Aminoquinolines. Antileishmanial Data.

Antileishmanial data against L. donovani in the hamster (Hanson, ref. 1) are shown in Table 1 for WR 6026 and 19 8-aminoquinoline analogs, 16 of which were prepared under this program and three under an earlier preparative contract (see footnote c). Portions of the data have been reported in the Journal of Medicinal Chemistry (ref. 2).

The first six compounds listed below WR 6026 are perhaps the most interesting. WR 226,292, a 5-methoxy derivative of WR 6026, approaches the high activity of the parent compound but with somewhat increased toxicity. All of the first four compounds possess a high toxicity (low minimum toxic dose) relative to SNL-07 (WR 239,374) and SNL-17 (WR 242,896), although the latter compounds are less active. SNL-07, which contains the cyclobutyl group in the side chain, has a G index of 188, and possesses a minimum toxic dose of 208 mg/kg/day(x4), four-fold better than WR 6026 in this respect. Modification of SNL-07 are recommended in future work to achieve a higher activity (G index > 188) coupled with the retention of the reduced toxicity.

In this connection, SNL-108, the 2,5-dimethoxy derivative of WR 6026 was prepared recently (3/84) for essentially the same reasons, i.e., retention of a reasonable activity coupled with reduced toxicity. If this approach proves fruitful, it is recommended that the 2,5-dimethoxy derivative of SNL-07 (WR 239,374) be prepared. L. donovani is a particularly refractory form of leishmaniasis and the 8-aminoquinolines have demonstrated the highest activity to date to our best knowledge.

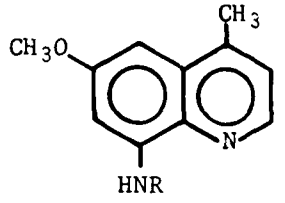
Phase 2. Other Aminoquinolines and Pyridines (6). Antileishmanial Data.

The six compounds prepared under the current contract are shown in the upper part of Table 2. These include three 7-aminoquinoline, SNL-22, 23 and 24, two 3-aminoquinolines, SNL-26 and 27, and one 4-aminopyridine with the WR 6026 side chain, SNL-25. None of these, however, showed significant activity in the Hanson L. donovani screen. Four other pyridine primaquine analogs, prepared under an antimalarial program, are shown at the bottom of Table 2. One of these, WR 228,769, a dimethoxy compound, has slight activity against L. donovani, giving 21% suppression of parasites at 208 mg/kg/day(x4). It is not clear, however, that this is sufficient activity to warrant further work in attempting to develop active pyridine analogs of WR 6026.

TABLE 1

ANTILEISHMANIAL ACTIVITY OF ANALOGS OF WR 6026

L. donovani, hamster (Hanson) (IM)



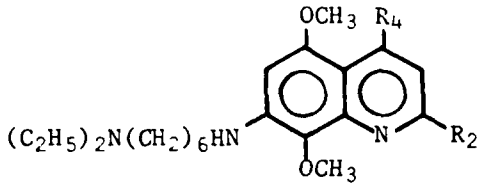
SNL No.	WR No.	R	Other Groups	Minimum Curative Dose % Suppression mg/kg/day(x4)	Min. Toxic Dose	G(a)
(b)	6,026	$-(CH_2)_6NEt_2$	-	34/0.05	52	474
(c)	226,292	$-(CH_2)_6NEt_2$	$5-OCH_3$	68/0.05	> 13	401
01	236,646	$-(CH_2)_6N(n-Pr)_2$	-	31/0.05	52	333
04	237,222	$-(CH_2)_6N(i-Pr)_2$	-	20/0.05	52	310
07	239,374	$-(CH_2)_6NH$(cycloBu)	-	99/0.81[d]	208	188
17	242,896	$-(CH_2)_6NEt_2$	$2-OCH_3$	56/0.81[d]	208	33
108	252,123	$-(CH_2)_6NEt_2$	$2,5-OCH_3$	(f)		
109	253,904	$-(CH_2)_6NEt_2$	$8-N'-CH_3$	(g)		
20	243,104	$-(CH_2)_5CH(CH_3)NH\cdot iPr$	-	97/0.2	13	~300
21	243,797	$-(CH_2)_5CH(CH_3)NEt\cdot iPr$	-	97/0.2	13	~300
(c)	223,756	$-(CH_2)_5NEt_2$	-	92/0.2	> 52	178
(c)	223,658	$-(CH_2)_7NEt_2$	-	73/0.2	52	97
02	236,917	$-(CH_2)_6N(Et)(CH_2CH_2NEt_2)$	-	78/0.81[d]	208	28
05	237,937	$-(CH_2)_6N(Et)(CH_2CH_2OH)$	-	79/13[d]	208	3
08	241,317	$-(CH_2)_6N(i-C_3H_7)_2$	$5-OCH_3$	100/13[d]	208	
09	241,318	$-(CH_2)_6N(n-Pr)_2$	$5-OCH_3$	100/13[d]	52	
13	242,474	$-(CH_2)_6NH(CH_2CH_2OH)$	-	99.9/13[d]	52	
06	238,872	$-(CH_2)_6NEt_2$	$6-SCH_3$	12/13[d]	>208	
14	242,784	$-(CH_2)_6NEt_2$	2-OH	63/208	>208	
03	237,067	$-(CH_2)_6N(n-Hex)_2$	No data			

Footnotes:
(a) Meglumine antimoniate, Glucantime index (IM), G = 1.
(b) Most active 8-Aminoquinoline, prepared WW II era; G = 708 orally.
(c) DADA17-73-C-3158, Third Annual Report, 1 July '75 to 30 June '76.
(d) Lowest dose tested.
(e) Replacing $6-OCH_3$.
(f) Recent submission.
(g) Submitted post-report under new contract DAMD17-84-C-4210.

TABLE 2

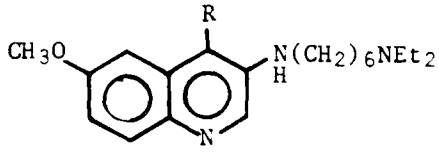
ANTILEISHMANIAL DATA. 7-AMINOQUINOLINES, 3-AMINOQUINOLINES AND SOME PYRIDINE ANALOGS (6)

(Hanson Test)



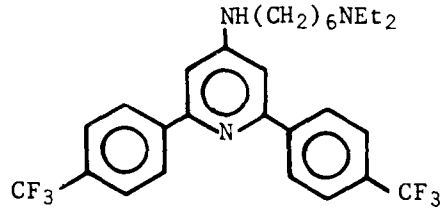
SNL No.	WR No.	R_2	R_4	% Suppression, Dose, mg/kg/dy(x4)	Min. Toxic Dose, mg/kg(x4)
22[a]	246,292	H	CH_3	26/208	>208
23	246,544	CH_3	CH_3	I/13	52
24	246,594	CH_3	H	I/52	208

(a) Also inactive against T. rhodesiense



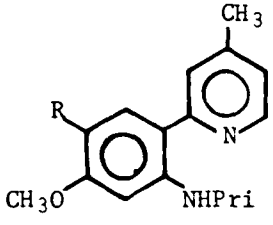
SNL-26, WR 247,190, R = CH_3
SNL-27, WR 247,208, R = H

Both inactive in the Hanson antileishmanial test.

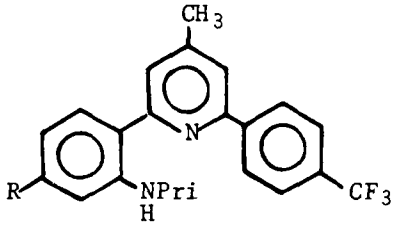


SNL-25, WR 246,594

Inactive in the Hanson antileishmanial test.



WR 230,216*, SN-270, R = H
WR 228,769*, SN-261, R = OCH_3
R = H, Inactive in the Hanson test
R = OCH_3, 21% suppression at 208 mg/kg



WR 220,549*, R = H
WR 249,699*, R = OCH_3
R = H, Inactive in the Hanson Test
R = OCH_3, data pending

*(Above four compounds prepared under DADA17-69-C-9065)

Phase 3. Aryl/Hetero Bis(amidoximes) and Bis(amidines). Antileishmanial Data.

Twenty of the title compounds were prepared and these are shown in Figure 1. In general the goal was to prepare both the bis(amidoxime) and the bis(amidine) of each structure but in two cases the preparation of both was considered too time-consuming. The rationale for the preparation of bis(amidines) is their effectivity as antitrypanocides, particularly T. rhodesiense, and, to a lesser extent, as leishmaniacides at the clinical level as discussed in section 1, Introduction. The rationale for preparing bis(amidoximes) is their greater water solubility, a factor of more importance in the case of larger complex structures which may possess a limited solubility in water and biological media.

The L. donovani data (Hanson) for the earlier 14 of the 20 phase 3 submissions are shown in Table 3, Part A, together with four commercial bis(amidines) and the antimonial Glucantime which serves as standard in the Hanson test, Part B (G index = 1).

Amongst the commercial bis(amidines), stilbamidine and pentamidine give comparable results in the Hanson test. Several of the phase 3 compounds show comparable activity but not greatly superior activity. Perhaps the best candidate phase 3 compounds are the two bis(amidoximes), SNL-36 and SNL-46, both monostyryl compounds (Fig. 1).

Drug	% Suppression (Hanson) Dose, mg/kg/day(x4) 208	52	13
Stilbamidine; WR 9,131	7/7T	65	40
Pentamidine; WR 4,931	6/6T	57	41
SNL-36 (AO); WR 249,238	74	63	44
SNL-46 (AO); WR 249,653	58	57	29

Thus the two bis(amidoximes) are significantly less toxic than the commercial bis(amidines) and the antileishmanial activity is roughly the same. The possibility does exist that one of the distyryl pairs, SNL-106 and SNL-107, will show distinctly higher activity when the data are in hand.

Several examples of activity against L. braziliensis are included in Table 3; in general the compounds are less active against this form of the leishmanial parasite.

Phase 4 Compounds. Commercial Drugs and their Bis(amidoxime) Analogs. Antileishmanial Data.

The structures of the phase 4 compounds are shown in Figure 2. The eight compounds are the bis(amidoxime)-bis(amidine) pairs of the four commercial drugs stilbamidine, pentamidine, berenil(diminazene) and

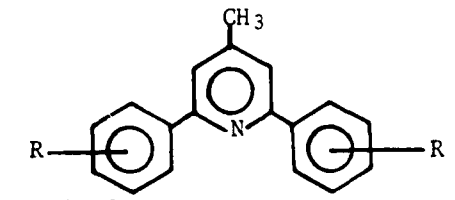
FIGURE 1

PHASE 3 STRUCTURES (20)

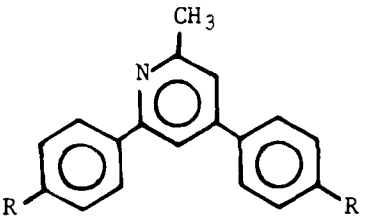
ARYL/HETERO BIS(AMIDOXIMES) AND BIS(AMIDINES)

(See also Figures 2 and 3 for related phase 4 and 5 structures)

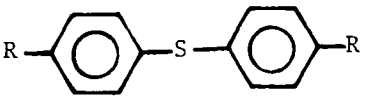
R = $-C(NH_2)=NOH$ (AO) and $-C(NH_2)=NH$ (AM) where AO = Amidoxime (first compound of pair) and AM = Amidine (second compound of pair)



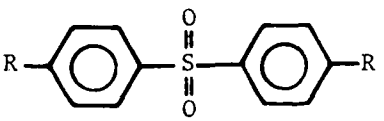
WR 248,396, SNL-28 ·2HCl; p,p; AO
WR 248,535, SNL-29 ·2HCl; p,p; AM
WR 249,942, SNL-49 ·2HCl; m,p; AO
WR 249,987, SNL-50 ·$2CH_3SO_3H$; m,p; AM
WR 250,043, SNL-51 ·$2CH_3SO_3H$; m,m; AO
WR 250,042, SNL-52 ·$2CH_3SO_3H$; m,m; AM



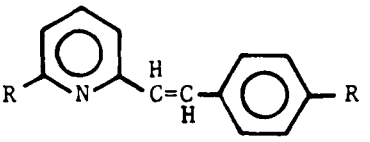
WR 250,261 SNL-53 ·$3CH_3SO_3H$; AO
WR 250,262 SNL-54 ·$3CH_3SO_3H \cdot 2H_2O$; AM



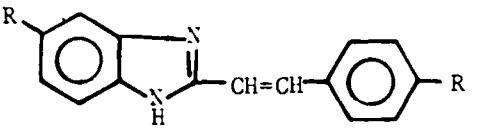
WR 251,842; SNL-100 ·$2CH_3SO_3H$; AO
WR 251,840; SNL-101 ·$2CH_3SO_3H$; AM



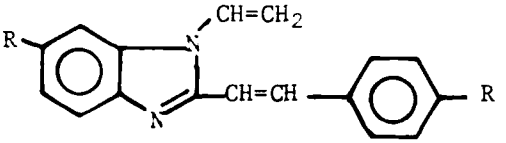
WR 251,843; SNL-103 ·$2CH_3SO_3H$; AO
WR 194,742; SNL-104 ·$2CH_3SO_3H$; AM



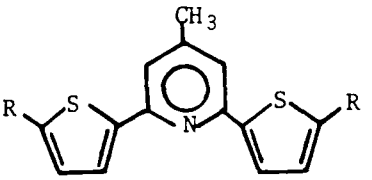
WR 249,238, SNL-36 ·$2CH_3SO_3H$; AO
WR 249,428, SNL-42 ·2HCl; AM



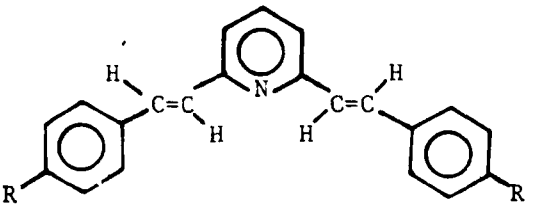
WR 249,653, SNL-46 ·$2CH_3SO_3H \cdot H_2O$; AO
WR 249,698, SNL-47 ·$2HCl \cdot H_2O$; AM



WR 249,754, SNL-48 ·$3CH_3SO_3H$ (R = AO)



WR 250,384, SNL-61 ·2HCl (R = AO)



WR 252,068, SNL-106 ·$3CH_3SO_3H \cdot H_2O$; AO
WR 252,070, SNL-107 ·$3HCl \cdot H_2O$; AM

TABLE 3

ANTILEISHMANIAL DATA, L. DONOVANI, HANSON (WR)

PHASE 3 BIS(AMIDOXIMES) AND BIS(AMIDINES)

SNL No.	WR or BN No.	Type Cpd	Parasite Suppression, %,[a] Dose, mg/kg/day (x4) 208(104)	52	13
	A. Candidate Drugs, Structures, Fig. 1				
	AO = Amidoxime and AM = Amidine				
28	248,396	AO	T	51	I
29	248,535	AM	T	T	T
36	249,238	AO	74,41[b](104)	63,32[b]	44
42	249,428	AM	T	T	56
46	249,653	AO	58	57	29
47	249,698	AM	T		40,T[b]
48	249,754	AO	T[b](104)	27[b]	
49	249,942	AO	T	36,40[b]	15
50	249,987	AM	T	78	37
51	250,043	AO	T	83,I[b]	43
52	250,042	AM	T	54,T[b]	I
53	250,261	AO		I[b]	
54	250,262	AM	T[b](104)	I[b]	
61	250,384	AO	30(208)[b] / 27(104)[b]	I / 25[b]	I
100	251,842	AO			
101	251,840	AM			
103	251,843	AO			
104	194,742	AM			
106	252,068	AO			
107	252,070	AM			
	B. Clinical Drugs; Structures, Fig. 2 (ref. 1)				
Glucantime (Sb^5) (Data variable)			95	-	27 13(3.25)
Stilbamidine Diisethionate			7/7T	65	40
Pentamidine Diiesethionate			6/6T	57	41
Berenil(Diminazene) Diaceturate[R]			7/7T	I	I
Hydroxystilbamidine Diisethionate			63	I	I

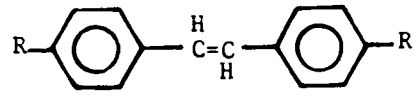
a) T = Toxic death, I = Inactive

b) L. braziliensis replacing L. donovani

FIGURE 2

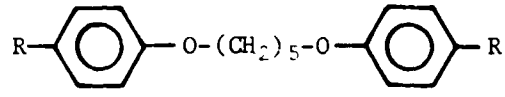
PHASE 4 STRUCTURES (8)

COMMERCIAL (CLINICAL) BIS(AMIDINES) AND THE CORRESPONDING BIS(AMIDOXIMES)



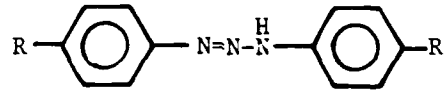
WR 250,257; BK 23958, SNL-55 $\cdot 2CH_3SO_3H$; AO
WR 9,131; BK 23976, SNL-56 $\cdot 2CH_3SO_3H$ (Stilbamidine)

WR 9131, Commercial Stilbamidine Diisethionate



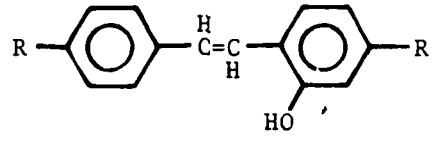
WR 250,385; BK 46353, SNL-63 $\cdot 2HCl \cdot 0.5H_2O$; AO
WR 4,931; BK 46362, SNL-64 $\cdot 2HCl$ (Pentamidine)

WR 4931, Commercial Pentamidine Diisethionate



WR 250,483; BK 50133, SNL-67 ·Dimaleate; AO
WR 27,800; BK 50142, SNL-68 ·Dimaleate (Diminazene; Berenil)

WR 27,800, Commercial Diminazene(Berenil) Diaceturate



WR 251,187; BK 63201, SNL-82 $\cdot 2HCl$; AO
WR 30,451 BK 63194, SNL-83 $\cdot 2HCl \cdot H_2O$; AM (2-Hydroxystilbamidine)

WR 30,457, Commercial 2-Hydroxystilbamidine Diisethionate

Available antitrypanosomal data are shown in Table 7.

hydroxystilbamidine. The primary purpose was to prepare, as stated earlier, the bis(amidoxime) analogs of these commercial drugs to determine if more active, less toxic products would result. It was worthwhile also to prepare the bis(amidine) in the same salt form for direct comparison.

The antileishmanial data are shown in Table 4, together with literature data (ref. 1) for the clinical drugs. In terms of L. donovani, data are available for the bis(amidoxime) analogs of both pentamidine and hydroxystilbamidine. Pentamidoxime is clearly more active than pentamidine against L. donovani. Similarly, hydroxystilbamidoxime is both somewhat more active and less toxic than hydroxystilbamidine.

Phase 5 Compounds. HOE 668 and Modifications. Antileishmania Data.

4-(4-Amidinophenoxy)benzaldehyde 4-amidinophenylhydrazone (HOE 668) was reported (1978, ref. 3) by W. Raether et al. of Hoechst AG to be superior to all known antileishmanial drugs. The reported results against L. donovani in the hamster were certainly impressive in that liver parasites were essentially 100% eradicated at the three dose levels under test, whereas this was not the case with three other antileishmanial drugs tested in parallel: Pentamidine, sodium stibogluconate and Glucantime. The test data were not reported in terms of a G index, as in the Hanson test, although it is clear that the compound is vastly more active than Glucantime based on the reported data. However, the drug's activity "was accompanied by a toxic effect on the large masses of the parenchyma in the liver and kidney....these toxic properties increase if the pulverized or dissolved compound is exposed to light for a short period of time" (ref. 4). Thus, the drug was dropped as clinical candidate.

In view of the potent leishmaniacidal activity of the drug in the hamster, it seemed worthwhile to modify the drug in an attempt to reduce the toxicity and retain all or a major part of its activity.

Accordingly, HOE 668 was resynthesized, together with the bis(amidoxime) analog, using a modified procedure, inasmuch as the Hoechst sequence (no experimental data) was unworkable in our hands. The structures of these two compounds and five others which were prepared are shown in Figure 3 and the available antileishmanial data are shown in Table 5.

Referring to Figure 3, SNL-84 is a Schiff's base modification of HOE 668 bis(amidoxime) which is probably hydrolytically unstable in vivo. SNL-93 and 96 are HOE 668 modifications in which the diphenyl oxide linkage is changed to a diphenyl thio linkage. Both of these changes were made in an effort to eliminate the photochemical instability of HOE 668.

SNL-97 and SNL-98 represent a structural pair which utilizes the phenoxy group of HOE 668. The structure is also a modification of 2,6-bis(amidoxime/amidine)-4-methylpyridines, SNL-28 and 29 (Fig. 1).

The only phase 5 data available at this writing is a two dose-level study of HOE 668 prepared under this program. If these data are compared with the Hoechst data in the same (similar) test system, there is a considerable discrepancy in the data and retesting is required.

TABLE 4

ANTILEISHMANIAL DATA, L. DONOVANI, HANSON (WR)

PHASE 4 BIS(AMIDOXIMES) AND BIS(AMIDINES) OF COMMERCIAL DRUGS

SNL No.	WR or BN No.	Type Cpd	Parasite Suppression, %,[a] Dose, mg/kg/day(x4) 208(104)	52	13
	Comparison Bis(amidoximes) with clinical Bis(amidines) (Prepared this program, same salt form, see Fig. 2)				
	1. Stilbamidine Pair. Dimethanesulfonates				
55	WR 250257 BK23958	AO	I(104)[b]	I[b]	
56	BK23976 WR 9131	AM	T(104)[b]	I[b]	
	2. Pentamidine Pair. Dimethanesulfonates				
63	WR 250385 BK46353	AO	T(52)[b]	89(26)	76
64	BK46362 WR 4931	AM	T(52)[b]	55(26)	39
	3. Berenil(Diminazene) Pair. Dimaleates				
67	WR 250,483 BK50133	AO			
68	BK50142 WR 27800	AM			
	4. 2-Hydroxystilbamidine Pair. Dihydrochlorides				
82	WR 251187 BK63201	AO	66(104)		24
83	BK63194 WR 30457	AM	49(104); 3/6TD		12
	Clinical Drugs; Structures, Fig. 2 (ref. 1)				
	Glucantime (Sb^5) (Data variable)		95(104)	-	26
	Stilbamidine Diisethionate		7/7T	65	40
	Pentamidine Diisethionate		6/6T	57	41
	Berenil(Diminazine) Diaceturate		7/7T	I	I
	Hydroxystilbamidine Diisethionate		63	I	I

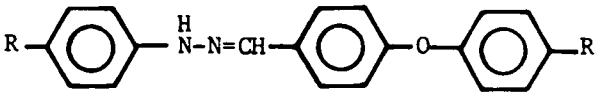
a) T = Toxic death, I = Inactive

b) L. braziliensis Panamensis replacing L. donovani

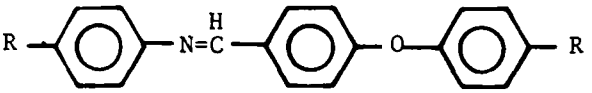
FIGURE 3

PHASE 5 STRUCTURES (7)

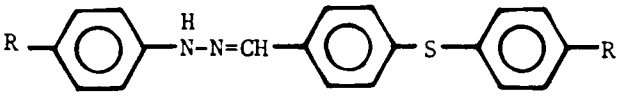
HOE 668 AND RELATED STRUCTURES



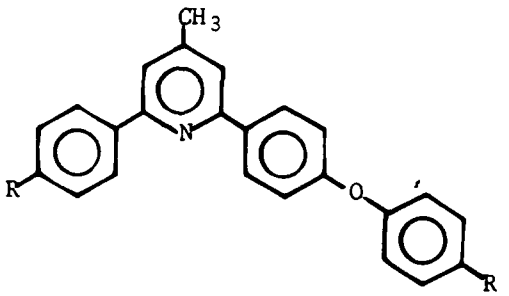
WR 250,574, SNL-76 ·Dimaleate; AO
WR 245,720, {SNL-77 ·2HCl·H_2O; AM
(HOE-668)



WR 251,232, SNL-84, free base; AO



WR 251,315, SNL-93 ·Dimaleate; AO
WR 251,336, SNL-96 ·2HCl·H_2O; AM



WR 251,781, SNL-97, free base; AO
WR 251,780, SNL-98, 3HCl·$3H_2O$; AM

TABLE 5

ANTILEISHMANIAL DATA, L. DONOVANI, HANSON (WR)

PHASE 5 BIS(AMIDOXIMES) AND BIS(AMIDINES). HOE-668 AND MODIFICATIONS

(Structures, see Fig. 3)

SNL No.	WR or BN No.	Type Cpd	Parasit Suppression, %[a]; Dose, mg/kg/day(x4) 104	25	13	3.3
76	250,574	AO				
77(b)	245,720	AM	6/6T		48	
HOE 668,	lit. ref. 3	AM		99.8	99.0	90.7
84	251,232	AO				
93	251,315	AO				
96	251,336	AM				
97	251,781	AO				
98	251,780	AM				

a) T = Toxic death, I = Inactive

b) HOE 668, prepared in this laboratory, BK57534.
The next line list data reported by Hoechst, ref. 3, for HOE 668.

2.2 ANTITRYPANOSOMAL DATA

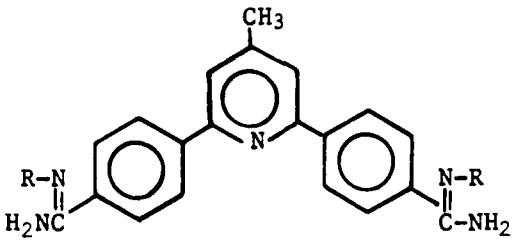
The available data for the phase 3,4 and 5 compounds against both T. rhodesiense (Africa) and T. cruzi (Central and South America) are shown in Tables 6,7 and 8, respectively.

Phase 3 Compounds. Bis(amidoximes) and Bis(amidines) (Table 6)

T. rhodesiense

Data for 18 of the 20 phase 3 compounds (administered SC) are shown in Table 6, Part A, and six of the compounds were tested also PO (oral). Of the 18 compounds administered SC, four show a minimum curative dose of less than 1.0 mg/kg. Of the six compounds administered orally, one (SNL-28) is active below 1.0 mg/kg.

The most interesting compound to date is SNL-28 (WR 248,396), 2,6-bis(4-amidoximinophenyl)-4-methylpyridine, which is curative at 0.83 mg/kg, both orally and S.C., and the minimum toxic doses P.O. and S.C. are at or above 424 mg/kg. The corresponding bis(amidine), SNL-29 (WR 248,535) is interesting also in that it has a minimum curative dose of 0.11 mg/kg (x1), SC, and retains a low toxicity, i.e., > 424 mg/kg (x1) against T. rhodesiense. However, it is less effective orally with a minimum curative dose of 13.3 mg/kg.



SNL-28, R = OH (AO), WR 248,396

SNL-29, R = H (AM), WR 248,535

T. cruzi (Chagas Disease)

As shown also in Table 6, some 13 compounds were tested against the refractory T. cruzi. None were active except SNL-101 (WR 251,840) which is minimally active at 40 mg/kg (Δ = 12.6 days). This compound contains a diphenyl thio group and it is of interest (perhaps) that another structure (SNL-96, Table 8) contains the same group and is curative (2/5C) at 640 mg/kg as discussed later. Some additional aryl sulfides might well be considered in future work.

TABLE 6

ANTITRYPANOSOMAL DATA. BIS(AMIDOXIMES) AND BIS(AMIDINES)

AO = Amidoxime, AM = Amidine

Phase 3 Compounds (Structures, Fig. 1)

SNL No.	WR or BN No.	Type Cpd.	Min. Cure Dose, mg/kg(x1) PO (Oral)	SC	Min. Toxic Dose, mg/kg(x1) PO	SC
		A. T. rhodesiense (Rand/Ager); mice				
28*	248,396	AO	0.83, 1.66	0.83	424	>424
29*	248,535	AM	13.3	0.11	>424	>424
36*	249,238	AO	6.65	6.65	>424	>424
42*	249,428	AM	6.65	1.66	>424	212
46*	249,653	AO	424.	6.65	>424	>424
47*	249,698	AM	26.5	0.21	>424	424
48	249,754	AO		3.33		>424
49*	249,942	AO		13.3		212
50	249,987	AM		13.3		106
51	250,043	AO		13.3		>424
52	250,042	AM		1.66		106
53	250,261	AO		6.65		212
54	250,262	AM		0.21		106
61	250,384	AO		26.5		424
100*	251,842	AO		26.5		106
101*	251,840	AM		13.3		106
103*	251,843	AO				
104	191,742	AM				
106*	252,068	AO		13.3		212
107*	252,070	AM		0.83 (a)		106
110	254,019	AO		26.5		>424
		B. T. cruzi (Chagas Disease), Ager; mice				

* Compounds marked with an asterisk were tested against T. cruzi; all were inactive except SNL-101 which was active at 40 mg/kg but toxic at 160 and 640 mg/kg.

(a) Lowest dose tested.

TABLE 7

ANTITRYPANOSOMAL DATA. COMMERCIAL BIS(AMIDINES) AND THE CORRESPONDING BIS(AMIDOXIMES).

AO = Amidoxime, AM = Amidine

Phase 4 Compounds (Structures Fig. 2)

SNL No.	WR or BN No.	Type Cpd.	Min. Cure Dose, mg/kg(x1) PO (Oral)	SC	Min. Toxic Dose, mg/kg(x1) PO	SC
			A. T. rhodesiense (Rane/Ager), mice			
			Stilbamidoxime and Stilbamidine, Dimethanesulfonates			
55*	250,257	AO		0.83		212
56	9,131	AM		0.42		106
			Pentamidoxime and Pentamidine, Dimethanesulfonates			
63	250,385	AO		0.83		106
64	4,931	AM		0.83		106
			Berenil (Diminazene), Dimaleates			
67	250,483	AO		0.11		212
68	27,800	AM		0.02, 0.01		106
			Hydroxystilbamidoxime and Hydroxystilbamidine, Dihydrochlorides			
82	251,187	AO		0.83		13.3, 26.5
83	30,457	AM		0.83, 1.66		53
			B. T. cruzi (Ager), mice			

* SNL-55. Toxic at 640 mg/kg and inactive at 160 and 40 mg/kg. No data for any other compounds.

TABLE 8

ANTITRYPANOSOMAL DATA. BIS(AMIDOXIMES) AND BIS(AMIDINES) HOE 668 (SNL-77) AND MODIFICATIONS

(Phase 5 Structures, Fig. 3)

SNL No.	WR or BN No.	Type Cpd.	Min. Cure Dose, mg/kg(x1) PO (Oral)	SC	Min. Toxic Dose, mg/kg(x1) PO	SC
		A. T. rhodesiense (Rane/Ager, mice)				
76	250,574	AO		6.65		>424
77	245,720	AM				
84	251,232	AO				
93	251,315	AO		13.3		>524
96	251,336	AM		0.83		212
97	251,781	AO		>424		>424
98	251,780	AM		26.5		424

B. T. cruzi (Chagas Disease, Ager, mice)

SNL No.	(Dose, mg/kg; 5 mice) 20	40	80	160	320	640
76						
77						
84		0/5C		4T		5T
93		I		I		2T
96		0/5C		2T;0/5C(a)		3T;2/5C(a)
97		I		I		I,0T
98		1T,I		3T,I		3T,I

C = Cure, T = Toxic death, I = Inactive

(a) Repeat test.

Phase 4 Compounds (Table 7)

T. rhodesiense

All of the commercial bis(amidines) and the corresponding bis-(amidoximes) show significant activity against T. rhodesiense when administered SC (no oral data) as shown in Table 7. Perhaps the most active drug is the berenil (diminazene) pair in which the bis(amidine) commercial drug (as the dimaleate) is curative at doses as low as 0.01 and 0.02 mg/kg. The corresponding bis(amidoxime) is also very active, being curative at 0.11 mg/kg and it is less toxic than the bis(amidine).

The triazene linkage in berenil may well be worth further exploitation in some of the active larger structures such as some of those shown in Figure 1, p. 8. Also the triazene linkage might be a useful replacement for the hydrazone linkage in HOE 668 (WR 245,270; Figure 3, p. 13).

T. cruzi (Chagas Disease)

Only one compound of the eight listed in Table 7 has been tested against T. cruzi. This is SNL-55 which is inactive. As discussed above under phase 3 compounds, the bis(amidines) as a class do not appear to be effective against T. cruzi although the data for berenil against T. cruzi might be of interest in view of the high activity against T. rhodesiense.

Phase 5 Compounds (Table 8)

T. rhodesiense

Data are available for five of the seven phase 5 compounds against T. rhodesiense as shown in Table 8. These are bis(amidoximes) and bis(amidines) so that the activity (SC) displayed by four of the five compounds is not surprising. The most active phase 5 compound to date is the bis(amidine) SNL-96. This is a modified HOE 668 in which the diphenyl ether group is replaced by a diphenyl sulfide group. SNL-96 (WR 251,336) has a minimum curative dose of 0.83 mg/kg when administered SC.

T. cruzi

As shown in Table 8, data are available for five compounds against the refractory T. cruzi. Again, essentially none are active which tends to confirm that bis(amidines) are not useful against T. cruzi. The diphenyl sulfide structure, SNL-96, was curative at 640 mg/kg in one test, but toxic in an earlier test such that the minimal activity may not be real. However, as noted earlier, SNL-101 (Figure 1, p. 8) is also a diphenyl sulfide bis(amidine) structure which displays minimal activity against T. cruzi at 40 mg/kg (Table 6, p. 16). Selected aryl sulfides might be worth exploring in future work.

2.3 References Cited. Section 2.

1) "Testing of Drugs for Antileishmanial Activity in Golden Hamsters infected with Leishmania donovani", W.L. Hanson, W.L. Chapman, Jr. and K.E. Kinnamon, International Journal for Parasitology, 7, 443 (1977).

2) "Analogs of WR 6026 as Antileishmanial Agents", M.P. LaMontagne, D.J. Dagli, M.S. Khan and P. Blumbergs, J. Med. Chem., 23, 981 (1980).

3) "Action of p(4-amidino-phenoxy)-benzaldehyde-p-amidino-phenyl-hydrazone dihydrochloride on Leishmania donovani infections in the golden hamster", W. Raether, H. Seidenath and H. Loewe, Ann. of Trop. Med. and Parasitology, 72, 543 (1978).

3. SYNTHESIS SUMMARY

Inasmuch as this is a Final Summary Report covering six and one-half years of work, no experimental work (with certain exceptions) will be included herein. Instead, the experimental procedures for the 56 target compounds will be referenced by the Annual Report number and page number as shown in Table 9. Referring to Table 9, each submission is listed by the SNL number representing the chronological sequence number according to the date of shipment to WRAIR. Also included for identification purposes is the ASI Code No. (Notebook No.), the Bottle Number and the Walter Reed (WR) number.

However, it is necessary that experimental details be presented and discussed herein for two groups of compounds, three each, which were not included in an Annual Progress Report. The first group includes three target compounds which were reported earlier in an Interim Progress Report covering the period 1 October 1978 to 31 December 1978.

SNL-08, MPL-XI-124, WR 241,317

SNL-09, MPL-XI-126, WR 241,318

SNL-13, DJD-I-125, WR 242,474

The second group are those reported in the last Quarterly Progress Report covering the period 1 January 1984 to 31 March 1984.

SNL-106, DJD-04-284, BN BK73832, WR 252,068

SNL-107, DJD-06-03, BN BK73841, WR 252,070

SNL-108, DJD-06-10, BN BK74384, WR 251,123

References cited are listed in experimental section 4.7.

3.1 8-(6-Diisopropylaminohexylamino)-5,6-dimethoxy-4-methylquinoline Dihydrochloride. SNL-08, WR 241,317

3.2 8-(6-Di-n-propylaminohexylamino)-5,6-dimethoxy-4-methylquinoline Dihydrochloride. SNL-09, WR 241,318

The essentially one-step reaction sequence for each of the above two side chain modifications of WR 6,026 (5-methoxy series) is shown in Chart No. 1. The condensation of 8-amino-5,6-dimethoxy-4-methylquinoline (ref. 1) with either 6-diisopropylaminohexyl chloride (ref. 2) or 6-di-n-propylaminohexyl chloride (ref. 2), followed by treatment with alcoholic hydrogen chloride afforded the target diamine dihydrochlorides, WR 241,317 and WR 241,318, respectively.

TABLE 9

TARGET COMPOUNDS SUBMITTED

Contract No. DAMD17-78-C-8001

SNL NO.	CODE NO.	BOTTLE NO.	WR NO.	ANNUAL NO.	REPORT PAGE
01	MPL-X-299	BH 49818	236,646	1	17
02	DCS-1-77B	BH 50802	236,917	1	18
03	MPL-IX-28	BH 56537	237,067	1	19
04	MS-1-200	BH 57098	237,222	1	20
05	DJD-I-30	BH 67432	237,937	1	21
06	DJD-I-62	BH 72353	238,872	1	23
07	DJD-I-89	BH 73903	239,374	1	23
08	MPL-XI-124	BH 84540	241,317	FSR	30
09	MPL-XI-126	BH 84531	241,318	FSR	31
13	DJD-I-125	BH 89492	242,474	FSR	32
14	DJD-I-160	BH 96755	242,784	2	16
17	DJD-I-171	BJ 01028	242,896	2	15
20	DJD-I-202	BJ 07486	243,704	2	17
21	MPL-XI-189	BJ 08198	243,797	2	19
22	KW-I-146	BJ 36870	246,292	2	20
23	KW-I-184D	BJ 39853	246,544	2	20
24	KW-I-200B	BJ 42467	246,594	2	21
25	KW-I-206A	BJ 42476	246,594	2	22
26	KW-I-264C	BJ 45824	247,190	3	11
27	KW-I-274C	BJ 46063	247,208	3	12
28	KW-II-98A	BJ 59006	248,396	3	14
29	KW-II-109A	BJ 63279	248,535	3	14
36	KW-II-178D	BJ 76285	249,238	3	15
42	KW-II-288B	BJ 84992	249,428	4	13
46	KW-III-71	BJ 92350	249,653	4	13
47	KW-III-91A	BJ 93599	249,698	4	13
48	KW-III-125B	BK 02584	249,754	4	15
49	MS-01-218	BK 15027	249,942	4	16

TABLE 9 Continued

SNL NO.	CODE NO.	BOTTLE NO.	WR NO.	ANNUAL NO.	REPORT PAGE
50	AM-VIII-16	BK 15607	249,987	4	16
51	KW-III-283A	BK 16917	250,043	5	25
52	AM-VIII-20	BK 16908	250,042	5	27
53	AM-VIII-24	BK 23912	250,261	5	29
54	DJD-04-41	BK 23967	250,262	5	30
55	AM-VIII-32	BK 23958	250,257	5	30
56	DJD-04-43	BK 23976	9,131	5	31
61	DJD-04-58	BK 46344	250,384	5	32
63	DJD-04-59	BK 46353	250,385	5	35
64	DJD-04-63	BK 46362	4,931	5	36
67	DJD-04-94	BK 50133	250,483	5	37
68	DJD-04-95	BK 50142	27,800	5	38
76	DJD-04-132(a)	BK 52084	250,574	6	28
76	DJD-04-131(b)	BK 52075	250,580	6	28
77	DJD-04-151	BK 57534	245,720	6	28
82	DJD-04-192	BK 63201	251,187	6	32
83	DJD-04-188	BK 63194	30,457	6	32
84	DJD-04-209	BK 63694	251,232	6	35
93	DJD-04-240	BK 64806	251,315	6	36
96	DJD-04-243	BK 65054	251,336	6	36
97	AM-VIII-56	BK 70082	251,781	6	40
98	AM-VIII-61	BK 70091	251,780	6	40
100	DJD-04-260	BK 70902	251,842	6	43
101	DJD-04-259	BK 70895	251,840	6	43
103	DJD-04-263	BK 70920	251,843	6	45
104	DJD-04-262	BK 71356	194,742	6	45
106	DJD-04-284	BK 73832	252,068	FSR	33
107	DJD-06-03	BK 73841	252,070	FSR	33
108	DJD-06-10	BK 74384	252,123	FSR	36

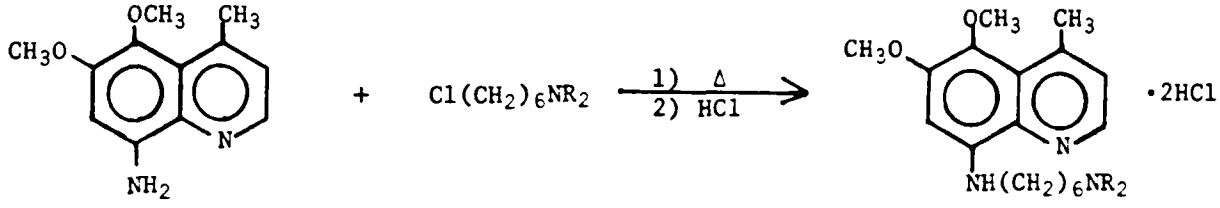
a) Dimaleate Salt, b) Free Base

Note: FSR = Final Summary Report, i.e., this report.

CHART NO. 1

8-(6-DIISOPROPYLAMINOHEXYLAMINO)-5,6-DIMETHOXY-4-METHYLQUINOLINE DIHYDROCHLORIDE. SNL-08, WR 241,317

8-(6-DI-N-PROPYLAMINOHEXYLAMINO)-5,6-DIMETHOXY-4-METHYLQUINOLINE DIHYDROCHLORIDE. SNL-09, WR 241,318



SNL-08, R = i-C_3H_7, WR 241,317

SNL-09, R = n-C_3H_7, WR 241,318

3.3 8-(6-Hydroxyethylaminohexylamino)-6-methoxy-4-methylquinoline Hydrochloride. SNL-13, WR 242,474

This side chain modification of WR 6,026 was prepared by the four-step synthesis shown in Chart No. 2. SNL-10, 11 and 12 are intermediates.

8-Amino-6-methoxylepidine was condensed with 1-bromo-6-phthalidimo-hexane (ref. 3) in the presence of diisopropylamine as acid acceptor to give the 6-phthalimidohexylaminoquinoline intermediate 1 (53%). The protecting phthalimido group was removed with hydrazine to afford the 8-diamine 2 (78%). This was condensed with ethyl bromoacetate to give the 8-(6-carboethoxymethylamino) precursor 3 (54%) after purification by column chromatography. The latter was reduced with lithium aluminum hydride and treated with aqueous alkali to give the title compound free base (68%). The free base was converted to the hydrochloride salt 4 (77%; 52% from 3) of the title target compound, WR 242,474.

The three target compounds prepared in the final quarter of this contract program are discussed below.

3.4 2,6-Bis(4-amidoximinostyryl)pyridine trimethanesulfonate. SNL-106, WR 252068

3.5 2,6-Bis(4-amidinostyryl)pyridine trihydrochloride. SNL-107, WR 252070

The reaction sequence used for the preparation of the above two compounds is shown in Chart No. 3.

2,6-Dimethylpyridine was condensed with 4-cyanobenzaldehyde in the presence of acetic anhydride to give the bis(styrylphenylnitrile) intermediate 1 (ref. 4). The structure of intermediate 1 was verified by an alternate synthesis route which gave an identical product (ref. 5).

A portion of intermediate 1 was treated with hydroxylamine to afford the bis(amidoxime) 2 as a free base which was allowed to react with methanesulfonic acid to give the target compound SNL-106 as the trimethane-sulfonate salt.

The corresponding target bis(amidine) 3 was obtained from the intermediate 1 using the standard Pinner reaction. The free base was isolated and converted to the target compound SNL-107 as the trihydro-chloride salt.

3.6 8-(6-Diethylaminohexylamino)-4-methyl-2,5,6-trimethoxyquinoline dihydrochloride. SNL-108, WR 252123

The reaction sequence for the title compound is shown in Chart No. 4. This 2-methoxyquinoline was prepared in view of the high activity of the parent 2-desmethoxy compound (WR 226,292) which possesses a Glucantime index of 401 in the Hanson leishmaniasis test, but possesses also a relatively high toxicity (Note: activity and toxicity data are shown in Table 1, Section 2). Further, while the introduction of a 2-methoxy

CHART NO. 2

8-(6-HYDROXYETHYLAMINO-1-HEXYLAMINO)-6-METHOXY-4-METHYLQUINOLINE DIHYDROCHLORIDE. SNL-13, WR 242,474

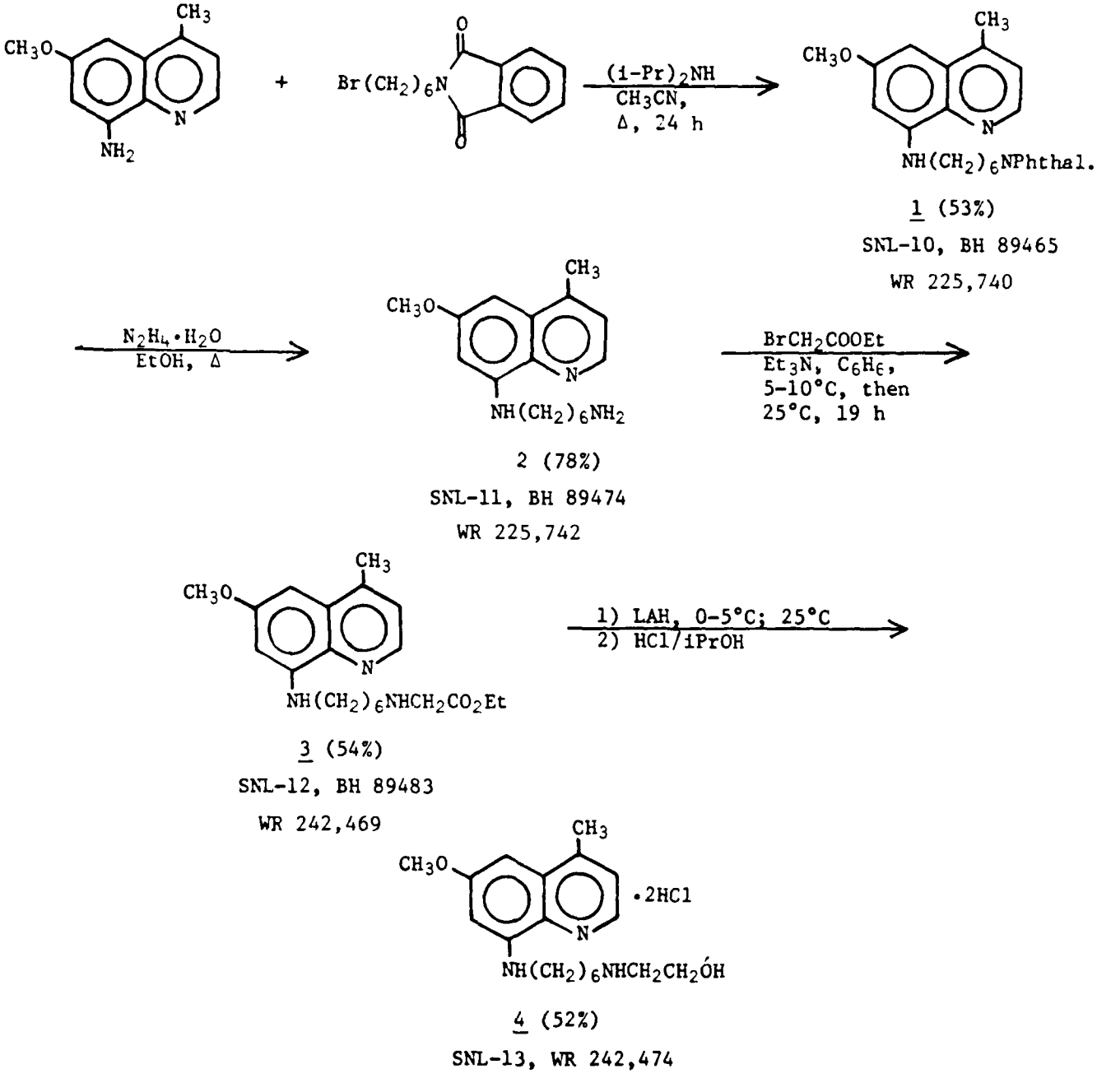
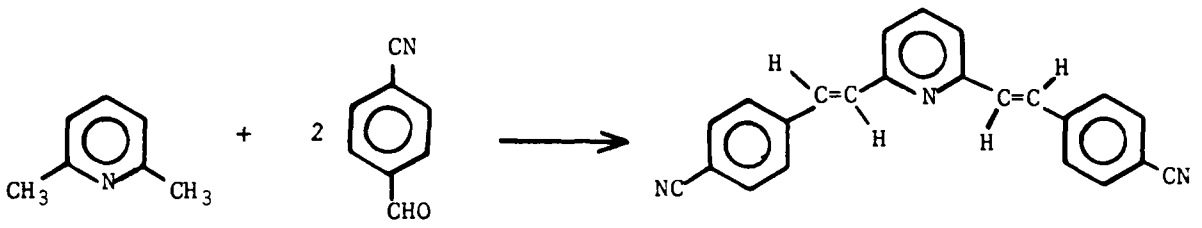


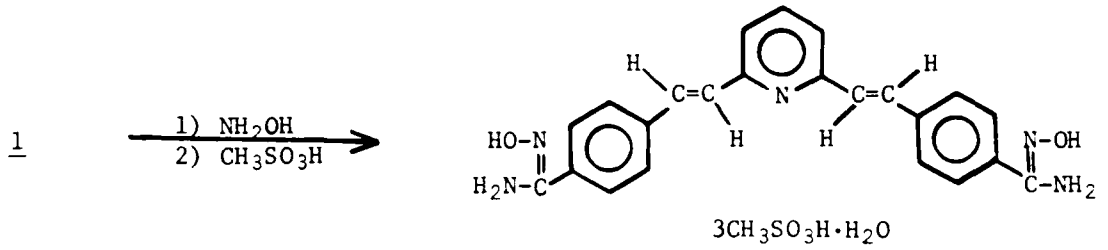
CHART NO. 3

2,6-BIS(4-AMIDOXIMINOSTYRYL)PYRIDINE TRIMETHANESULFONATE MONOHYDRATE, SNL-106

2,6-BIS(4-AMIDINOSTYRYL)PYRIDINE TRIHYDROCHLORIDE MONOHYDRATE, SNL-107

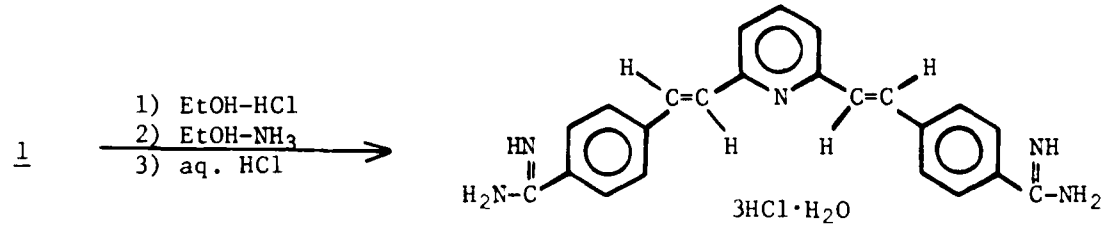


1 (24%)



$3CH_3SO_3H \cdot H_2O$

2, SNL-106 (63%), BK 73832
WR 262,068

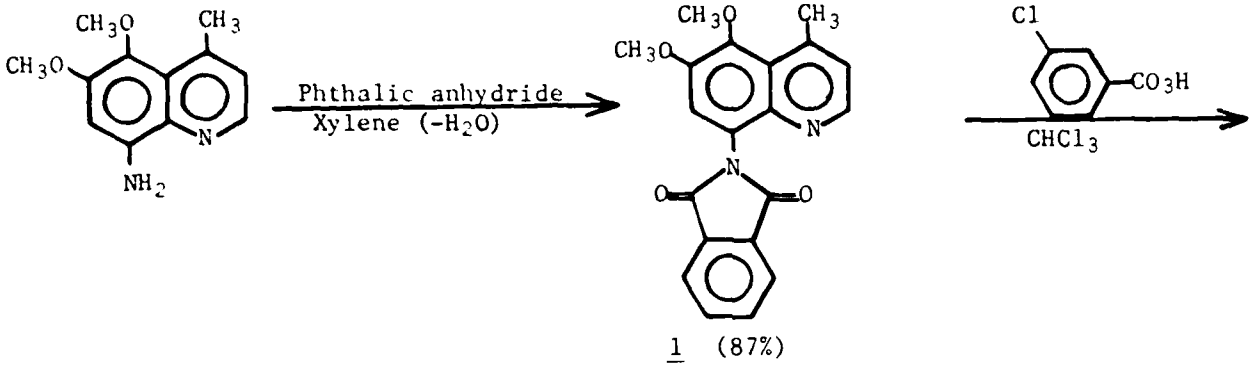


$3HCl \cdot H_2O$

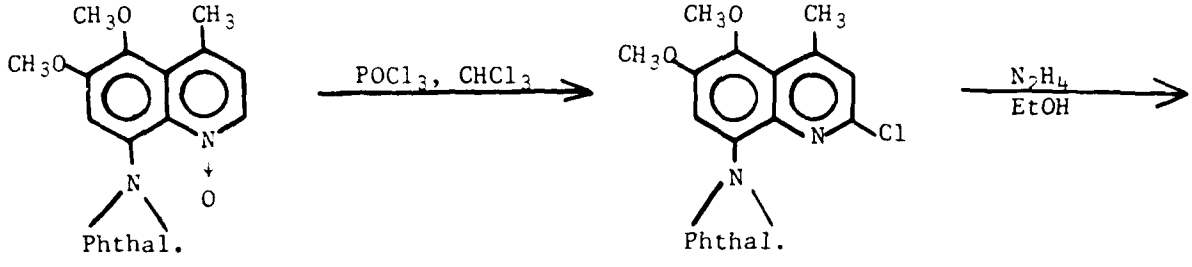
3, SNL-107, BK 73841, WR 262,070
(37%)

CHART NO. 4

8-(6-DIETHYLAMINOHEXYLAMINO)-4-METHYL-2,5,6-TRIMETHOXYQUINOLINE DIHYDROCHLORIDE. SNL-108, WR 252,123

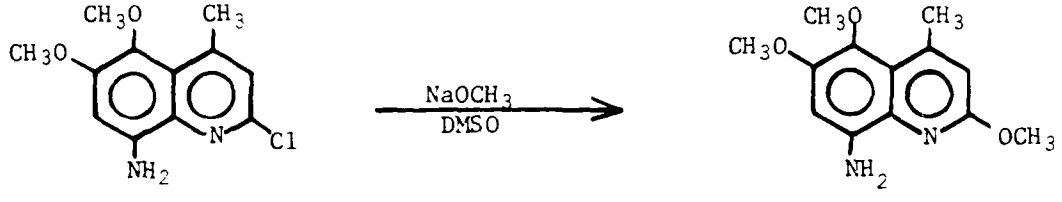


1 (87%)



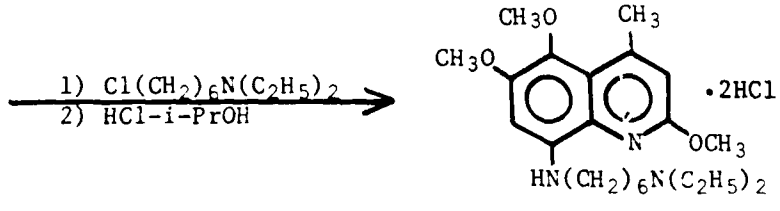
2 (77%)

3 (76%)



4 (92%)

5 (54%)



6 (54%), SNL-108, BK 74384

WR 252,123

group into WR 6,026 to form WR 242,896 does reduce the activity markedly (from G = 474 to G = 33), the toxicity is four-fold less. Thus, the purpose of preparing the title compound was to reduce the toxicity of WR 6,026 while retaining a large fraction of its antileishmanial activity.

The synthesis sequence (Chart No. 4) starts with 8-amino-5,6-dimethoxy-4-methylquinoline, an available intermediate used in the synthesis of a number of active antimalarials. Thereafter the synthesis follows the same procedures used for the preparation of SNL-17 (WR 242,896), the corresponding 5-desmethoxy analog (ref. 6, p. 15) as well as 2,6-dimethoxy-4-methylprimaquine (WR 238,605).

4-Methyl-5,6-dimethoxy-8-aminoquinoline was treated with phthalic anhydride to give the protected amine 1 which was oxidized to the N-oxide 2 with 3-chloroperbenzoic acid. Treatment of 2 with phosphoroxychloride afforded the 2-chloro derivative 3 which was deprotected with hydrazine to give the 8-aminoquinoline 4. Treatment of 4 with sodium methoxide gave the 2-methoxy-8-aminoquinoline 5. In the final step, intermediate 5 was allowed to react with the side chain reagent diethylaminohexyl chloride to afford the target compound 6 as a free base which was submitted as the target dihydrochloride salt, SNL-108, WR 252,123.

4. EXPERIMENTAL

All melting points and boiling points are uncorrected. Infrared spectra were recorded using a Perkin-Elmer 237B Spectrometer. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana. Vapor phase chromatography was performed using an F and M Model 810 with a flame ionization detector. NMR spectra, when required, were determined on a Varian Model T60 Spectrometer. All thin layer chromatography was carried out using Brinkmann Instruments, Inc., 0.25 mm silica gel plates with a fluorescent indicator (Polygram Sil G/UV_{254}) unless otherwise stated.

Experimental data are presented herein for two groups of compounds, three each, which were not included in one of the Annual Progress Reports. The first group includes three target compounds which were reported earlier in an Interim Progress Report covering the period 1 October 1978 to 31 December 1978.

SNL-08, MPL-XI-124, WR 241,317

SNL-09, MPL-XI-126, WR 84,531

SNL-13, DJD-I-125, WR 242,474

The second group are those reported in the last Quarterly Progress Report covering the period 1 January 1984 to 31 March 1984.

SNL-106, DJD-04-284, BN BK73832, WR 252,068

SNL-107, DJD-06-03, BN BK73841, WR 252,072

SNL-108, DJD-06-10, BN BK74384, WR 252,123

4.1 8-(6-Diisopropylaminohexylamino)-5,6-dimethoxy-4-methylquinoline Dihydrochloride. SNL-08, WR 241,317

The one-step reaction sequence is shown in Chart No. 1.

A mixture of 8-amino-5,6-dimethoxylepidine (ref. 1, 10 g, 0.046 mol) and 6-diisopropylaminohexyl chloride (ref. 2, free base, 14.0 g, 0.064 mol) was heated at 130-135°C for 16 h and cooled to room temperature. The reaction mixture was dissolved in warm chloroform (500 mL) and treated with ammonium hydroxide (9 mL) to pH 9. The organic layer was separated, dried (K_2CO_3) and concentrated to a brown residue. This was chromatographed over silica gel (800 g, EM Labs) and eluted with 1% MeOH:$CHCl_3$ (3 L) and 2% MeOH:$CHCl_3$ (3 L) to remove starting material and impurities. Additional impurities were removed by elution with 5% MeOH:$CHCl_3$ (1 L) and 12.5% MeOH:$CHCl_3$ (2 L) to afford crude alkylation product (7.3 g). Additional product (2.2 g) was isolated with 15% MeOH:$CHCl_3$ (1 L) and 20% MeOH:$CHCl_3$ (1 L). These two fractions were combined and rechromatographed over silica gel (200 g, EM Labs) and eluted with 5% MeOH:$CHCl_3$ (2 L) and 7.5% MeOH:$CHCl_3$ (1 L) which on concentration gave a dark residue.

This residue was dissolved in anhydrous ether (500 mL) and extracted with 10% KOH solution (50 mL). The aqueous layer was separated and reextracted with ether (2 x 100 mL). The combined ether layers were dried over anhydrous potassium carbonate and concentrated to give the title free base (7.07 g). The base (7.0 g) was dissolved in 2-propanol (20 mL) and HCl/2-propanol (10.3 mL, 3.07 N) was added followed by ether (57 mL). The solid was filtered and washed with ether-2-propanol and ether to yield 5.8 g, mp 104.5-106.5°C. This material was recrystallized from 2-propanol (20 mL) and ether (400 mL) to yield pure title compound, 5.3 g (23%), mp 107-109°C.

Anal. Calcd for $C_{24}H_{39}N_3O_2 \cdot 2HCl \cdot H_2O$ (492.6): C, 58.52; H, 8.79; Cl, 14.40; N, 8.53; O, 9.75. Found: C, 58.67; H, 8.67; Cl, 14.00; N, 8.36; O, 9.79.

4.2 8-(6-Di-n-propylaminohexylamino)-5,6-dimethoxy-4-methylquinoline Dihydrochloride. SNL-09, WR 241,318

The one-step reaction sequence is shown in Chart No. 1.

A mixture of 8-amino-5,6-dimethoxylepidine (10 g, 0.046 mol) and 6-dipropylaminohexylchloride (ref. 2, free base, 13.0 g, 0.059 mol) was heated at 105-115°C for 24 h and cooled to room temperature. The reaction mixture was dissolved in warm chloroform (500 mL) and treated with ammonium hydroxide (15 mL) to adjust the pH to 9. The organic layer was dried (K_2CO_3) and concentrated. The dark residue was chromatographed over silica gel (750 g, EM Labs) and eluted with chloroform (2 L) and 2.5% MeOH:$CHCl_3$ (2 L) to remove starting material (2.1 g). Additional impurities were removed when eluted with 5% MeOH:$CHCl_3$ (1 L). Elution with 7.5% MeOH:$CHCl_3$ (2 L) afforded impure alkylation product (7.0 g). Pure alkylation product (5.0 g) was isolated when eluted with 10% MeOH:$CHCl_3$ (1 L) and 12.5% MeOH:$CHCl_3$ (1 L). The impure alkylation product was purified by rechromatography over silica gel. The purified alkylation product was dissolved in anhydrous ether (700 mL) and extracted with 10% aqueous KOH (200 mL). The aqueous layer was separated and reextracted with ether (2 x 150 mL). The combined ether layers were dried over anhydrous potassium carbonate and concentrated to yield a dark colored oil (10.8 g). The oil was dissolved in warm 2-propanol and treated with HCl/2-propanol solution (15.9 mL, 3.06 N). Concentration afforded a red gummy residue which was treated with charcoal in warm 2-propanol (150 mL). The volume of solvent was reduced to ∿ 20 mL and treated with anhydrous ether (200 mL). The product initially oiled and later solidified to give a red solid, mp 90-93°C (7.8 g). The crude salt (6.8 g) was recrystallized from 2-propanol (44 mL) and ether (200 mL) to afford pure title compound, 3.6 g (16%), mp 96.5-98°C.

Anal. Calcd for $C_{24}H_{39}N_3O_2 \cdot 2HCl \cdot H_2O$ (492.6): C, 58.52; H, 8.79; Cl, 14.40; N, 8.53. Found: C, 58.49; H, 8.58; Cl, 14.60; N, 8.31.

4.3 8-(6-Hydroxyethylaminohexylamino)-6-methoxy-4-methylquinoline Hydrochloride. (SNL-13, WR 242,474)

This was prepared by the four-step synthesis shown in Chart No. 2. SNL-10, 11 and 12 are intermediates.

8-(6-Phthalimidohexylamino)-6-methoxylepidine (SNL-10, WR 225,740): A mixture of 8-amino-6-methoxylepidine (23.7 g, 0.126 mol), 6-bromo-1-phthalimidohexane (ref. 3, 39 g, 0.126 mol) and diisopropylamine (17.7 mL, 12.8 g, 0.126 mol) in acetonitrile (200 mL) was refluxed for 24 h after which time additional 6-bromo-1-phthalimidohexane (39 g, 0.126 mol) and diisopropylamine (17.7 mL, 12.8 g, 0.126 mol) were added. The mixture was refluxed for an additional 48 h, cooled to room temperature, poured into water (1000 mL) and extracted with chloroform (3 x 500 mL). The combined organic layers were dried (K_2CO_3) and concentrated to a dark residue which was chromatographed over silica gel (700 g, EM Labs) and eluted with chloroform (5 L), collecting small fractions (250 mL). The product-containing fractions were combined and concentrated to a thick oil which was dissolved in hot 2-propanol (500 mL) and cooled to room temperature to yield the title compound, 28 g (53%), mp 96-97°C.

Anal. Calcd for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.06. Found: C, 71.74; H, 6.32; N, 9.95.

8-(6-Aminohexylamino)-6-methoxylepidine (SNL-11, WR 225,742): A mixture of 8-(6-phthalimidohexylamino)-6-methoxylepidine (25.1 g, 0.06 mol) and hydrazine hydrate (75%, 12.5 mL) in ethanol (600 mL) was refluxed for 5 1/2 h and cooled to room temperature. The solid phthalyl hydrazide was filtered and triturated with methylene chloride (300 mL). The filtrate on concentration gave additional phthalyl hydrazide which was triturated with methylene chloride (500 mL) and filtered. The filtrate was extracted with 20% sodium hydroxide solution (200 mL). The aqueous layer was separated and reextracted with methylene chloride (2 x 100 mL). The combined organic layers were dried (K_2CO_3) and concentrated to give the title compound, 13.4 g (78%), mp 56-58°C. An analytical sample was prepared by recrystallization from petroleum ether (60-110°C), mp 56-58°C.

Anal. Calcd for $C_{17}H_{25}N_3O$: C, 71.04; H, 8.77; N, 14.62. Found: C, 70.86; H, 8.77; N, 14.39.

8-(6-Carbethoxymethylaminohexylamino)-6-methoxylepidine (WR 242,469 SNL-12): To a cold solution (5-10°C) of 8-(6-aminohexylamino)-6-methoxylepidine (13.4 g, 0.047 mol) and triethylamine (4.7 g) in distilled benzene (150 mL), was added over a period of 45 min ethyl bromoacetate (7.6 g, 0.046 mol) in distilled benzene (50 mL). The reaction mixture was allowed to warm to room temperature and stirred for 19 h. The reaction mixture was filtered to remove triethylamine hydrobromide. The filtrate on concentration gave crude product which was chromatographed over silica gel (250 g, EM Labs) and eluted with chloroform (4.5 L) to remove the major by-product (diester, 1.7 g). Elution with 1% and 2% MeOH:$CHCl_3$ (1.2 L each) afforded the

product, mp 50-52°C (9.5 g, 54%). An analytical sample was prepared by recrystallization from petroleum ether (60-110°C), mp 51-53°C.

Anal. Calcd for $C_{21}H_{31}N_3O_3$: C, 67.53; H, 8.37; N, 11.25. Found: C, 67.28; H, 8.14; N, 11.06.

8-(6-Hydroxyethylaminohexylamino)-6-methoxy-4-methylquinoline Hydrochloride. SNL-13, WR 242,474: To a cold (0-5°C) suspension of $LiAlH_4$ (2.6 g, 0.068 mol) in dry tetrahydrofuran (THF, 250 mL) was added over a period of 1 h 8-(6-carbethoxymethylaminohexylamino)-6-methoxylepidine (8 g, 0.021 mol) in dry THF (100 mL). The reaction mixture was warmed to room temperature and stirred for 3/4 h. After cooling to 0-5°C, methanol (40 mL) was added carefully followed by 10% potassium hydroxide (40 mL). The orange-colored mixture was filtered (celite) and the solid was washed with THF (100 mL) and anhydrous ether (200 mL). The combined organic layers were concentrated and the residue was redissolved in methylene chloride (300 mL) and dried (K_2CO_3). Concentration afforded a solid (6.1 g) which was recrystallized twice from acetonitrile to give light colored product, 4.2 g (68%), mp 83-85°C.

Anal. Calcd for $C_{19}H_{29}N_3O_2$: C, 68.85; H, 8.82; N, 12.68. Found: C, 68.86; H, 8.69; N, 12.81.

The above free base (4.5 g, 0.015 mol) was dissolved in hot 2-propanol (15 mL) and cooled to room temperature. To this solution was added HCl/ 2-propanol (4.0 mL, 3.06 N) followed by anhydrous ether (15 mL). Filtration afforded the title hydrochloride salt, 4.3 g (77%), mp 117-119°C.

Anal. Calcd for $C_{19}H_{29}N_3O_2 \cdot HCl$: C, 62.02; H, 8.22; Cl, 9.65; N, 11.42. Found; C, 61.79; H, 8.04; Cl, 9.58; N, 11.22.

4.4 2,6-Bis(4-amidoximinostyryl)pyridine trimethanesulfonate monohydrate SNL-106, BK73832, WR 252,068

4.5 2,6-Bis(4-amidinostyryl)pyridine trihydrochloride monohydrate SNL-107, BK73841, WR 252,072

The sequence to the title compounds is shown in Chart No. 3.

2,6-Bis(4-cyanostyryl)pyridine (1): - A solution of 4-cyanobenzaldehyde (100 g, 0.76 mol) and 2,6-dimethylpyridine (40 g, 0.37 mol) in distilled acetic anhydride (325 mL) was heated to reflux for 18 h (oil bath, 170-175°C). The acetic acid and acetic anhydride were removed by distillation at atmospheric pressure until the kettle temperature reached 215°C (oil bath, 245°C). The viscous oil solidified upon cooling to ~ 50°C. This dark brown solid was mixed with deionized water (750 mL) and heated (steam bath, internal temperature 88-90°C) for 1 h. The mixture was cooled and the water layer was removed by decantation and discarded. The residue was treated with aq. potassium carbonate (500 mL) at ~ 50°C for 15 min. The resulting gummy solid was collected by filtration, washed with water (2 x 200 mL) and titurated with hot ethanol (1 L) (steam bath) for 15 min.

The mixture was filtered to give a yellow green solid, 35 g, mp 148-150°C. Second crop material, 14 g, mp 140-155°C, was obtained from the mother liquor; the total crude yield was 49 g.

The first crop (35 g) was dissolved in dichloromethane (1.2 L), charcoaled twice, filtered (celite) and concentrated to near dryness. The residue was diluted with petr. ether (35-60°C, 1 L) to give a solid, 32 g, which was triturated with hot ethanol (400 mL) for 15 min. The mixture was filtered and the solid was washed with petr. ether (250 mL) to give an off-white product, 28 g (88% recovery), mp 170-172°C. This material was chromatographed over silica gel, (200 g, EM Labs) eluting with chloroform (1.5 L). The eluate was concentrated to give again an off-white solid, ∿ 28 g, with no change in melting point. An additional 8.1 g of the same purity was obtained from the second crop material. The combined yield at this point was 36.1 g (29%), mp 170-172°C.

To improve the purity, this material (∿ 28 g), together with product from an earlier probe run (4.1 g), were combined and dissolved in hot 2-methoxyethanol (500 mL). The mixture was charcoaled, filtered (celite) and the volume was reduced to ∿ 200 mL. The solution was diluted with ethanol (100 mL) and the volume reduced again to ∿ 200 mL. The off-white solid was collected by filtration, washed with petr. ether (250 mL) and dried (50°C, 0.3 mmHg, 18 h) to give pure title compound 1, 26.7 g (24%), mp 173-174°C; lit. 175-176°C, ref. 4.

Anal. Calcd for $C_{23}H_{15}N_3$ (333.37): C, 82.86; H, 4.54; N, 12.60. Found: C, 82.71; H, 4.43; N, 12.72.

2,6-Bis(4-amidoximinostyryl)pyridine trimethanesulfonate monohydrate (2) SNL-106: - A solution (∿ 50°C) of hydroxylamine hydrochloride (15.6 g, 0.22 mol) in methanol (240 mL) was added to a warm solution (∿ 60°C) of the bis(nitrile) 1 (12 g, 0.036 mol) in pyridine (120 mL). Sodium bicarbonate powder (18.8 g, 0.22 mol) was added portionwise to the clear yellow solution over 10 min and the mixture was refluxed for 3.5 h (steam bath, internal temperature 71°C). The mixture was filtered while hot and the collected solid was washed with ethanol (2 x 100 mL). The light yellow green solid was slurried with deionized water (2 x 1 L) and with ethanol (1 L). The solid was collected by filtration, washed with anhydrous ether (2 x 250 mL) and dried (25°C, 0.3 mmHg, 20 h) to give the title compound as a free base, 10.1 g (70%), mp 250-252°C dec.

An analytical sample was obtained by recrystallization of the free base (1 g) from DMF-ethanol. The melting point was unchanged. The analysis is shown below.

Anal. Calcd for $C_{23}H_{21}N_5O_2$ (399.43): C, 69.15; H, 5.30; N, 17.53. Found: C, 68.91; H, 5.37; N, 17.27.

The unrecrystallized free base (7.8 g, 0.018 mol) was suspended in warm methanol (400 mL) and the pH was adjusted with methanesulfonic acid to ∿ 1. The clear yellow solution was filtered (celite), concentrated to dryness (aspirator) and the gummy residue was washed with anhydrous ether (3 x 250 mL). The residue was dried at 0.3 mmHg for 5 min and dissolved in warm ethanol (250 mL). The volume of ethanol was reduced to ∿ 100 mL. Additional ethanol (150 mL) was added and the volume was reduced to ∿ 200 mL (aspirator, steam bath). At this point, a bright yellow solid precipitated and additional methanesulfonic acid (∿ 20 drops) was added to maintain pH of the solution at ∿ 1. The mixture was stirred for 2 h. The bright yellow solid was collected by filtration, washed with anhydrous ether (250 mL) and dried (25°C, 0.1 mmHg, 16 h) to give crude title compound, 13 g (94%), mp 212-214°C dec. This material (13 g) was dissolved in hot 10% aqueous ethanol (130 mL), charcoaled, filtered (celite) and the filtrate was diluted with ethanol (200 mL). The volume was reduced to ∿ 150 mL and additional ethanol (150 mL) was added. When the volume was reduced to ∿ 200 mL (steam bath, aspirator) a bright yellow solid precipitated. The mixture was stirred for 2.5 h while maintaining the pH at ∿ 1 with additional methanesulfonic acid (∿ 20 drops). The bright yellow solid was collected by filtration, washed with ethanol (2 x 150 mL) and dried (25°C, 0.3 mmHg, 18 h) to give pure title bis(amidoxime) trimethanesulfonate salt 2, 12.5 g (90% conversion, 63% yield), mp 227-230°C dec. Of this 11.0 g was shipped to WRAIR on March 23, 1984 as SNL-106, Code No. DJD-04-284, BK73832, WR 252,068.

Anal. Calcd for $C_{23}H_{21}N_5O_2 \cdot 3CH_3SO_3H \cdot H_2O$ (705.77): C, 44.24; H, 5.00; N, 9.92; S, 13.63. Found: C, 44.47; H, 5.01; N, 10.05; S, 13.42.

2,6-Bis(4-amidinostyryl)pyridine trihydrochloride monohydrate (3) SNL-107:- A suspension of the bis(nitrile) 1 (10 g, 0.030 mol) in anhydrous alcohol (300 mL) was saturated with hydrogen chloride gas for 1 h at 0°C (ice-bath). The mixture was stirred at room temperature for seven days in a tightly-stoppered flask. The resulting bright yellow suspension was concentrated to dryness at ∿ 40°C (water bath) under reduced pressure (aspirator) and treated with anhydrous ether (500 mL). The resulting bright yellow solid was collected by filtration, washed with anhydrous ether (100 mL) and added to saturated ethanolic ammonia (800 mL). The mixture was stirred at room temperature for seven days. The mixture was concentrated to dryness and dissolved in hot dilute hydrochloric acid (400 mL; concd $HCl:H_2O$ = 1:9). The solution was filtered while hot, cooled to room temperature and stirred for 1 h to give a bright yellow solid (20 g, wet), mp > 300°C. This solid was dissolved in hot 30% aq ethanol (500 mL). The solution was filtered (celite) and cooled to room temperature. Concd hydrochloric acid (2 mL) was added to the yellow solution and a bright yellow solid precipitated. The mixture was stirred for 2 h and filtered to give the title compound 3, first crop, 6.89 g. A second crop, 3.89 g, was obtained from the mother liquor.

The first crop material (6.8 g) was dissolved in hot water (65 mL), filtered (celite), rinsing the pad with water (10 mL). Concd hydrochloric acid (∿ 10-12 drops) was added to the filtrate to induce crystallization. The solution was stirred at room temperature for 2 h. The resulting bright yellow solid was collected by filtration, washed with cold water (2 x 10 mL) and dried (25°C, 0.3 mmHg, 18 h) to give pure title compound, mp > 300°C, 5.45 g (37%).

Anal. Calcd for $C_{23}H_{21}N_5 \cdot 3HCl \cdot H_2O$ (494.84): C, 55.82; H, 5.30; Cl, 21.50; N, 14.15. Found: C, 56.07; H, 4.19; Cl, 21.62; N, 14.28.

A 6.0 g sample was shipped to WRAIR on March 23, 1984 as Code No. DJD-06-03, BK 73841. This sample included material (1.0 g) of equal quality from a probe run, together with 5.0 g from the batch described above.

4.6 8-(6-Diethylaminohexylamino)-4-methyl-2,5,6-trimethoxyquinoline Dihydrochloride. SNL-108, BK 74384, WR 252,123

The sequence is shown in Chart No. 4.

5,6-Dimethoxy-4-methyl-8-phthalimidoquinoline (1): - 5,6-Dimethoxy-4-methyl-8-nitroquinoline (50 g) was obtained from current contract DAMD17-83-C-3208 as Lot Nos. DAG-III-281 and IV-128. This was hydrogenated over Raney Nickel to give 8-amino-5,6-dimethoxy-4-methylquinoline (90%), mp 98-100°C.

A mixture of the latter (39.5 g, 0.18 mol) and phthalic anhydride (26.8 g, 0.18 mol) in xylene (900 mL) was refluxed for 2.5 h while water was removed with a Dean-Stark trap. After cooling to room temperature, a light brown solid separated which was collected and triturated with hot ethanol (1 L). The mixture was filtered and the grey solid was dried (25°C, 0.3 mmHg, 16 h) to give the title compound, 55 g (87%), mp 228-230°C. An analytical sample, mp 230-232°C, was prepared by recrystallization from 2-methoxyethanol.

Anal. Calcd for $C_{20}H_{16}N_2O_4$ (348.35): C, 68.95; H, 4.63; N, 8.04. Found: C, 68.66; H, 4.82; N, 8.17.

5,6-Dimethoxy-4-methyl-8-phthalimidoquinoline N-oxide (2): - A solution of the 8-phthalimidoquinoline 1 (55 g, 0.16 mol) in hot chloroform (2.5 L) was charcoaled and filtered (celite) to give a light yellow solution. 3-Chloroperoxybenzoic acid (56 g, 85%, 0.27 mol) was added and the solution was refluxed (steam bath) for 2 h. Additional 3-chloroperoxybenzoic acid (20 g, 85%, 0.1 mol) was added and the solution was refluxed for an additional 1 h. The mixture was stirred at room temperature overnight (18 h), extracted with 10% aq potassium carbonate (2 x 1.5 L) and with water (1 L) and dried (K_2CO_3). The volume of the chloroform solution was reduced to ∿ 1 L and chromatographed over aluminum oxide, basic (850 g, J.T. Baker, Brockman Activity Grade 1), eluting with chloroform (7 L). Concentration of the

eluate gave a yellow-orange solid which was triturated with ethanol (100 mL). The solid was collected by filtration, washed with additonal ethanol (2 x 15 mL), petr. ether (2 x 100 mL) and dried (25°C, 0.3 mmHg, 16 h) to give the title N-oxide 2 as an off-white solid, 44.2 g (77%), mp 230-232°C. A mixed mp and TLC (Brinkmann, Polygram Sil G/UV_{254}, 5% MeOH:$CHCl_3$) indicated the product was different than starting material. This material was used as such in the next step without further purification.

2-Chloro-5,6-dimethoxy-4-methyl-8-phthalimidoquinoline (3): - Phosphorus oxychloride (40 mL, 65.8 g, 0.43 mol) was added dropwise over 15 minutes to a warm (∿ 50°C) solution of the N-oxide 2 (44.2 g, 0.12 mol) in chloroform (1.1 L). A mild exotherm maintained a gentle reflux of the chloroform solution during the addition. The solution was refluxed (steam bath) for 1 h. Additional phosphorus oxychloride (4 mL, 6.6 g, 0.04 mol) was added to the mixture and refluxed for an additional 30 min. The mixture was cooled to room temperature and poured over crushed ice (2 L). The pH was adjusted to ∿ 10-11 with 20% aq sodium bicarbonate (1 L) and dried ($MgSO_4$). Concentration of the solution gave a dark residue which was triturated with warm ethanol (50 mL). The mixture was filtered and the precipitate was washed with additional ethanol (2 x 10 mL), ether (100 mL) and dried (25°C, 0.3 mmHg, 2 h) to give crude title compound, 37.6 g (81%), mp 190-192°C dec.

The crude product was chromatographed over silica gel (400 g, EM Labs), eluting with chloroform (2 L). Concentration of the eluate gave pure title compound 3, 35.2 g (94% recovery, 76% yield), mp 193-195°C dec. An analytical sample was prepared by recrystallization from 2-methoxyethanol with no change in mp.

Anal. Calcd for $C_{20}H_{15}ClN_2O_4$ (382.81): C, 62.75; H, 3.95; Cl, 9.26; N, 7.32. Found: C, 62.29; H, 4.05; Cl, 9.44; N, 7.54.

8-Amino-2-chloro-5,6-dimethoxy-4-methylquinoline (4):- Anhyd hydrazine (6 g, 0.19 mol) was added to a warm solution of intermediate 3 (35.2 g, 0.09 mol) in ethanol (2 L). The mixture was refluxed (steam bath) for 75 min, concentrated to dryness (aspirator) and the residue was slurried with dichloromethane (MDC, 1.5 L) for 15 min. The mixture was filtered and the solid was reslurried with additional MDC (0.5 L) for 15 min. The mixture was filtered again and the combined filtrate was washed with 20% aq sodium hydroxide (750 mL) and with water (1 L) and dried (K_2CO_3). Concentration of the MDC solution gave a yellowish green solid which was slurried with petr. ether (250 mL) for 1 h. The solid was collected by filtration and dried (25°C, 2 h) to give the title compound 4, 21.4 g (92%), mp 134-136°C. An analytical sample, mp 135-137°C, was prepared by recrystallization from benzene-hexane (1:2).

Anal. Calcd for $C_{21}H_{13}ClN_2O_2$ (252.70): C, 57.03; H, 5.18; Cl, 14.03; N, 11.09. Found: C, 57.25; H, 5.30; Cl, 14.22; N, 11.26.

8-Amino-4-methyl-2,5,6-trimethoxyquinoline (5): - Freshly-cut sodium metal (2.9 g, 0.13 mol) was dissolved in dry methanol (110 mL, dried over magnesium turnings and stored over molecular sieves (type 3A) under a nitrogen atmosphere. The solvent was removed (aspirator) and the residual sodium methoxide was dried (0.1 mmHg, ∿ 50°C) for 20 min. Distilled dimethylsulfoxide (150 mL) was added to the dry sodium methoxide under nitrogen atmosphere at 95-100°C (oil bath). The 2-chloro intermediate 4 (21.4 g, 0.08 mol) was added to the mixture and the internal temperature was maintained between 90-95°C for 1 h. The mixture was cooled to room temperature and poured carefully with stirring into a mixture of saturated brine (500 mL) and crushed ice (1 L). The mixture was stirred for 30 minutes, the resulting light-yellow green solid was collected by filtration, washed with cold water (1 L), dissolved in ether (1 L), dried (K_2CO_3), charcoaled and filtered (celite). The volume of the solvent was reduced to ∿ 100 mL (aspirator) and diluted with petr. ether (200 mL). The solid was collected by filtration, washed with petr. ether (100 mL) and dried (25°C, 2 h) to give the title 2-methoxy intermediate 5 (16.4 g, 78%), mp 113-115°C.

This material was dissolved in anhyd ether (250 mL), charcoaled and the mixture was filtered (celite). The volume of the solvent was reduced (aspirator) to ∿ 150 mL, cooled and placed in the refrigerator (16 h). The light-yellow green solid was collected by filtration, washed with ether (2 x 50 mL) and dried (25°C, 0.3 mmHg, 2 h) to give pure title compound 5, 11.3 g (69% recovery, 54% yield), mp 114-115°C.

Anal. Calcd for $C_{13}H_{16}N_2O_3$ (248.28): C, 62.88; H, 6.50; N, 11.29. Found: C, 62.63; H, 6.65; N, 11.09.

8-(6-Diethylaminohexylamino)-4-methyl-2,5,6-trimethoxyquinoline Dihydrochloride (6) SNL-108: - Concd ammonium hydroxide (30 mL) was added to a suspension of 6-diethylaminohexyl chloride hydrochloride (20 g, 0.09 mol, Lot ASI-SH-04-72) in ether (300 mL). The organic layer was separated, dried (K_2CO_3) and concentrated to give 6-diethylaminohexyl chloride (16.2 g, 96% recovery).

A mixture of 8-amino-4-methyl-2,5,6-trimethoxyquinoline (8 g, 0.03 mol), 6-diethylaminohexyl chloride (12 g, 0.06 mol) and sodium bicarbonate (5.35 g, 0.06 mol) was heated under nitrogen atmosphere at 140-145°C for 5.5 h. Additional 6-diethylaminohexyl chloride (4 g, 0.02 mol) and sodium bicarbonate (1.85 g, 0.02 mol) were added and heating was continued for an additional 7 h. After cooling to room temperature, the product mixture was triturated with anhyd ether (2 x 250 mL) and filtered. The organic layer was concentrated to give a light-brown thick oil (16.9 g) which was dissolved in petr. ether (100 mL) and cooled (dry ice-acetone bath). The mixture was filtered cold and the collected solid was washed with cold (dry ice-acetone) petr. ether (25 mL). The solid was dissolved in anhyd ether. The petr. ether filtrate (125 mL) was cooled (dry ice-acetone bath), the mixture was filtered and this solid was dissolved also in anhyd ether. Both ether solutions were combined and concentrated to give 6.3 g of thick oil. The petr. ether filtrate (ca 125 mL) was concentrated

to give 10.5 g of thick oil which was chromatographed over silica gel (200 g, J.T. Baker, 60-200 mesh), eluting with anhyd ether (3 L). Appropriate fractions were combined and concentrated to give the title compound as a free base, 4.7 g, as a thick yellow oil (solidifies upon cooling). Similarly the 6.3 g of oil from the ether solutions was chromatographed over silica gel (120 g) and eluted with anhyd ether to give additional product, free base, 4 g, as a thick yellow oil. Overall, a total of 8.7 g (67%) of the title compound as a free base was obtained from the reaction mixtures.

The free base of the title compound (8.7 g, 0.02 mol) was dissolved in 2-propanol (50 mL) and the solution was adjusted to pH ∿ 1-2 with hydrogen chloride-saturated 2-propanol (∿ 5 mL). The solvent was removed (aspirator) at 40-50°C (water bath) and the gummy residue was redissolved in 2-propanol (∿ 50 mL). Most of the solvent was removed (aspirator), and the gummy residue was triturated with anhyd ether (100 mL). The crude salt was collected by filtration, washed with anhyd ether (50 mL) and air-dried (∿ 15-20 min) to give the crude title compound as an off-white solid, ∿ 10 g, mp 151-153°C (eff). This material was dissolved in 2-propanol (50 mL). The solution was filtered and diluted with anhyd ether (100 mL) and stirred at room temperature for 1 h. The solid was collected, washed with anhyd ether (3 x 50 mL) and dried to give the title compound 6 as a colorless solid, 5.6 g (54%), mp 160-161°C (eff). Of this 5.0 g was shipped to WRAIR on April 5, 1984 as Code No. DJD-06-10, WR 252,123.

Anal. Calcd for $C_{23}H_{37}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$ (485.47): C, 56.89; H, 8.30; Cl, 14.60; N, 8.65. Found: C, 56.83; H, 8.66; Cl, 14.29; N, 8.36.

A sample was dried at 120°C for a short time by the analyst and the compound analyzed acceptably as anhydrous material.

Anal. Calcd for $C_{23}H_{37}N_3O_3 \cdot 2HCl$ (476.47): C, 57.97; H, 8.25; Cl, 14.99; N, 8.82. Found: C, 57.67; H, 8.49; Cl, 14.72; N, 8.63.

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